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Potential for Milk Containing Penicillin G or Amoxicillin to Cause Residues in Calves

J.M.B. Musser,* K. L. Anderson,*
J. E. Rushing,† and W. A. Moats‡
*Department of Farm Animal Health and Resource Management,
College of Veterinary Medicine and
†Department of Food Science,
College of Agriculture and Life Sciences
North Carolina State University, Raleigh 27606
‡Meat Science Research Laboratory, United States Department of Agriculture,
Agricultural Research Service, Beltsville, MD 20705

ABSTRACT

The potential for antibiotic residues in calves from consuming milk containing penicillin G or amoxicillin was investigated. Six calves were fed milk replacer, 6% body weight twice daily, containing 0.293, 2.92, or 5.85 μg of penicillin/ml (ppm) G or 0.25, 1.0, or 2.0 μg of amoxicillin/ml for three consecutive feedings. Urine and blood samples were collected after each feeding. Serum and urine samples were tested with a microbial receptor assay and a microbial growth inhibition assay to indicate potential drug residues. Penicillin G and amoxicillin were detected in the serum and urine of several calves 3 h after drinking spiked milk replacer. Possible violative drug residues in the calves were detected by the microbial growth inhibition assay up to 15 h after drinking spiked milk replacer. Penicillin G, but not amoxicillin, could be detected in urine 24 h after the final feeding of spiked milk replacer. Subsequently, six calves were fed milk replacer containing 11.7 μg of penicillin G/ml (ppm) twice daily, 6% body weight per feeding. Calves were slaughtered 3 h after the final feeding. Mean (±SD) concentrations of penicillin G measured by high-pressure liquid chromatography in liver, kidney, muscle, and serum were 0.409 (±0.167) μg/g, 0.031 (±0.012) μg/g 0.008 (±0.002) μg/g, and 0.013 (±0.006) mg/ml, respectively. This study indicates that calves fed milk with amoxicillin or penicillin G could possibly have violative residues if slaughtered within 24 h after feeding. Violative drug residues in liver tissue were found in calves slaughtered 3 h after consuming milk replacer containing 11.7 μg of penicillin G/ml (ppm).
(**Key words:** penicillin, amoxicillin, residues, calves)

Abbreviation key: $\mathbf{B/B_0}$ = counts per minute of sample divided by counts per minute of a negative control sample, **LOD** = limit of detection, **MGIA** = microbial growth inhibition assay, **MRAM** = microbial receptor assay method, **penG** = penicillin G, **PPG** = procaine penicillin G.

INTRODUCTION

Food safety is an important issue for the dairy and beef industries. A primary concern of consumers regarding meat quality and safety is drug residues in meat (Sundlof, 1993). This public awareness and concern have made it crucial that marketed meat contains no drug residues. Veal calves have one of the highest incidences of violations of drug residues for any meat animal category (Norcross and Brown, 1991; Salisbury et al., 1989; van Dresser and Wilcke, 1989). Also, analysis of violative drug residues for the years 1993, 1994, and 1995 showed that bob veal calves were the most common source of violative drug residues in meat, and penicillin was the most frequent drug causing residue violations (Paige and Pell, 1997).

On dairy farms, calves may be fed milk from cows treated with antibiotics that have been withheld from the bulk tank (Heinrichs et al., 1995). Research has indicated this milk, termed "waste" milk by Chik et al. (1975) can be an effective feed for calves, providing good weight gains with no adverse health effects (Chardavoyne et al., 1979; Chik et al., 1975, Kesler, 1981; Yndestad and Helmen, 1980). The economic benefits of feeding pasteurized waste milk have been investigated (Jamaluddin et al., 1996). However, the issue of drug residues was not mentioned, even though pasteurization has minimal effects on the destruction of penG (Moats, 1999). The cause of some drug residue violations has been identified as a result of feeding calves milk containing antibiotics (Guest and Paige, 1991; van Dresser and Wilcke, 1989). Feeding colos-

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Corresponding author: J.M.B. Musser; e-mail: jmusser@usaid.gov.

trum containing drug residues has also been identified as a potential source in some drug residue violations (Paige and Pell, 1997).

Yndestad and Helmen (1980) detected penicillin (≤0.95 IU/ml) in the serum of calves fed milk and milk replacer containing procaine penicillin G (**PPG**) at concentrations achievable after treating a cow via the intramuscular or intramammary routes with PPG. Prange et al. (1984) detected penicillin G (**penG**) in the urine of calves that consumed milk replacer fortified with PPG at levels to simulate approximately 25 and 50% recovery of antibiotic in the milk following intramammary infusion of 100,000 IU of PPG. However, with a microbial growth inhibition assay (**MGIA**), residues of penG were not detected in the kidney or muscle of these calves at slaughter. The study did not utilize a quantifiable analytical method to measure drug concentrations in the tissues.

The objective of this study was to determine the presence of antibiotic residues in urine and serum and to use these results as an indicator of potential drug residues in calves following consumption of milk replacer containing PPG and amoxicillin. HPLC was used to assay tissue and serum to confirm the drug residue status of the calves.

MATERIALS AND METHODS

Determination of penG and Amoxicillin in Serum and Urine by Microbial Receptor Assay Method (MRAM)

The MRAM (Charm II, Charm Sciences Inc., Malden, MA) was performed according to the manufacturer's instructions (Charm II test for β-lactams in serum and tissue using tablet reagents—operator's manual. Charm Sciences Inc.). The MRAM was assessed and validated for the determination of penG and amoxicillin in serum and urine.

Precision. Serum and urine samples spiked with penG at concentrations of 0, 0.006, 0.015, 0.03, and 0.06 μg/ml or with amoxicillin at concentrations of 0, 0.01, 0.025, 0.05, and 0.10 μg/ml were tested on five different days during a 2-wk period. The mean for each concentration, expressed as $\mathbf{B/B_0}$ (where B = counts per minute of the sample and B_0 = counts per minute of a negative control sample), was plotted against the known concentrations of samples. A quadratic equation was obtained using B/B_0, the dependent variables, and the known concentration for that sample, the independent variables.

Accuracy. Serum and urine samples containing known concentrations of penG or amoxicillin were tested in a blind trial. Four concentrations (0, 0.006, 0.015, and 0.03 μg/ml or 0, 0.01, 0.025, and 0.05 μg/ml of penG or amoxicillin, respectively) of penG or amoxicillin in serum and urine were tested a total of six times for each concentration in each matrix. Test tubes were labeled alphabetically and 3 ml of urine with the appropriate penG or amoxicillin concentration was dispensed per tube. The concentration of penG or amoxicillin corresponding to the letter designation was not revealed to the technician performing the tests. Results of the assays were plotted as known versus measured penG or measured amoxicillin concentrations.

Between-day precision. Serum and urine samples spiked to achieve concentrations of 0, 0.006, 0.015, and 0.03 μg of penG/ml of serum and urine or 0, 0.01, 0.025, 0.05, and 0.10 μg of amoxicillin/ml of serum and urine were assayed on 5 consecutive days. The spiked serum and urine samples were made daily. Results of daily runs, B/B_0, were analyzed using analysis of variance.

Limit of detection (LOD). The LOD was determined as three standard deviations from the mean B/B_0 value for the zero concentration, in accordance with manufacturer's recommendations (Charm II test for beta-lactams in serum and tissue using tablet reagents—operator's manual. Charm Sciences Inc.).

Cross-reactivity. An antibiotic was added to control serum and urine samples containing 0.015 μg of penG/ml. The antibiotic added was either cloxacillin at 33.333 μg/ml, sulfadimethoxine at 100.0 μg/ml, oxytetracycline at 100.0 μg/ml, or erythromycin at 100.0 μg/ml in each sample. A total of 10 samples were run for each combination of drugs, including control serum samples containing 0.015 μg of penG/ml. The B/B_0 was used to interpret data from these tests.

An antibiotic was added to serum and urine samples containing 0.025 μg of amoxicillin/ml. The antibiotic added was either cephapirin at 1.0 μg/ml, sulfadimethoxine at 1.0 μg/ml, oxytetracycline at 1.0 μg/ml, or erythromycin at 1.0 μg/ml in each sample. A total of 10 samples were run for each combination of drugs, including control serum samples containing 0.025 μg of amoxicillin/ml. Also, penG was added to achieve 0.006 μg of penG/ml of serum to samples containing 0.01 μg of amoxicillin/ml of serum; 10 samples were run with this concentration. Ten serum samples containing only 0.01 μg of amoxicillin/ml and 10 serum samples containing only 0.006 μg of penG/ml were run. The B/B_0 was used to interpret data from these tests.

Liquid Chromatography

The concentrations of penG in serum, muscle, kidney, and liver were determined at the Meat Science Research Laboratory, Agricultural Research Service, USDA by the automated liquid chromatograpy method

developed by Moats and Romanoski (1998). Recoveries at 0.01 μg/ml and 0.10 μg/ml were 91 and 80%, respectively. The LOD was 0.005 μg/ml (or μg/g).

Lack of a chromatographic peak at the proper time provided unequivocal evidence that penG was not present above the detection limit of the analytical method. Low levels of penG were confirmed by analyzing a replicate after treatment with β-lactamase (β-lactamase, Charm II, Charm Sciences Inc.) (Moats and Romanoski, 1998).

Animals

Six male dairy calves, 2 to 4 d old, with a prior history of not receiving antibiotic treatments were purchased from a state dairy farm. The adequacy of passive transfer was determined by a commercial test (BOVA-S, VMRD, Inc., Pullman, WA) and by measuring total proteins with a refractometer. A urine sample from each calf was tested by the MRAM for the presence of β-lactam antibiotics and the MGIA (Live Animal Swab Test, Editek, Inc., Burlington, NC) according to manufacturer's recommendations (Performing the live animal swab test: a self-instructional guide. 9/89. Editek, Inc., Burlington, NC) for the presence of antimicrobials. The calves were housed in individual stalls (Research Facility, College of Veterinary Medicine, NCSU) and fed milk replacer (Kid Milk Replacer, Purina Mills, Inc., St. Louis, MO) at 12% BW daily in two feedings a day. The milk replacer tested negative for the presence of β-lactam antibiotics using a microbial receptor assay (Anderson et al., 1997). Calves had an acclimation period of 2 wk before the study started. During this period, calves were trained to drink milk from buckets. Neither grain supplement nor hay was provided. Fresh water was provided ad libitum.

Experiment 1: Antibiotic Residues in Urine and Serum Determined by MGIA and MRAM

PPG was added to reconstituted milk replacer to achieve concentrations of 0.5, 5.0, and 10.0 μg/ml, equivalent to 0.293, 2.93, and 5.85 μg/ml (ppm) penG, respectively. Amoxicillin was added to reconstituted milk replacer to achieve concentrations of 0.25, 1.0, and 2.0 μg/ml (ppm). Although the concentrations of penG and amoxicillin in milk fed to calves is quite variable dairy to dairy, the concentrations used in this investigation are in a range of concentrations that have been measured in milk from treated cows (Anderson et al., 1998; Ang et al., 1997; Schaffer and McGuffey, 1980). Calves were fed twice daily, 6% BW per feeding, for three consecutive feedings. The feedings occurred at 0600 and 1500 h on d 1 and at 0600 on d 2 (6:00 a.m., 3:00 p.m., and 6:00 a.m., respectively). The calves were fed milk replacer with added β-lactam antibiotic for three consecutive feedings with the following exception; milk replacer with PPG at 0.5 μg/ml and amoxicillin at 2.0 μg/ml were fed for only one feeding. Normal nonspiked milk replacer was fed during the interval between trials. The interval between feeding different levels of PPG or amoxicillin was a minimum of 4 d to allow sufficient time for drug elimination from the calf. Urine and serum samples had to test negative prior to the start of a new dosage or drug trial using the MRAM and MGIA.

Urine samples were collected at 0, 3, and 9 h following the first feeding, 15 h after the second feeding, and 24 h after the final feeding of milk replacer containing the β-lactam antibiotic (Tables 1 and 2). The samples were tested for the β-lactam antibiotic with the MGIA and MRAM. If antibiotic is detected in urine by the MGIA, a positive MGIA indicates that the animal may have violative drug residues present in tissues (Performing the live animal swab test: a self-instructional guide. 9/89. Editek, Inc., Burlington, NC).

Blood samples were collected at 0, 3, 6, and 9 h following the first, 15 h after the second, and 24 h following the final feeding of milk replacer containing the β-lactam antibiotic (Tables 1 and 2). The blood samples were allowed to clot while refrigerated, centrifuged (1400 × *g* for 10 min), the serum decanted, and tested for the β-lactam antibiotic with the MRAM.

Experiment 2: Confirmation of PPG Residues in Tissue by HPLC

Calves were fed milk replacer with PPG added to a concentration of 20 μg/ml, equivalent to 11.7 μg of penG/ml (ppm). This concentration was determined as a result of experiment 1 and to approximate the higher pen G concentrations measured in milk after treating cows with clinical mastitis with 100,000 IU penG intramammary infusion per affected quarter (Anderson 1998). Calves were fed twice daily, 6% BW per feeding, for five consecutive feedings. The feedings occurred at 0600 and 1500 h each day (6:00 a.m. and 3:00 p.m., respectively). Urine and blood were collected at 0, 3, and 9 h following the first and third feedings and 15 h after the second and fourth feeding of spiked milk replacer (Table 3). Calves were euthanized, by captive bolt, 3 h after the fifth feeding of spiked milk replacer, which was 51 h after the start of the trial. Blood samples were collected immediately, placed on ice, allowed to clot, and centrifuged (1400 × *g* for 10 min), and serum was collected. Samples of kidney, liver, and muscle (semitendinosus/semimembranosus) were collected. A minimum of 500 g of tissue was cut into 2.5-

Table 1. Detection of penicillin G in serum and urine of calves after being fed milk replacer containing 0.293, 2.93, and 5.85 μg/ml (ppm) of penicillin G (procaine penicillin G at 0.5, 5.0, and 10.0 μg/ml, respectively). Serum was assayed by the microbial receptor assay method (MRAM). The urine was assayed using the microbial growth inhibition assay (MGIA) and MRAM. The table indicates the number of individual calf serum or urine samples that had detectable levels of penicillin G by MRAM and the number of individual calf urine samples that had positive MGIA results. A positive MGIA signifying a possible violative drug residue in the animal.

Sampling time after feeding (h)	Time of feeding spiked milk replacer[3]	0.293 μg/ml[1]			2.93 μg/ml[2]			5.85 μg/ml[2]		
		Serum	Urine		Serum	Urine		Serum	Urine	
		MRAM	MGIA	MRAM	MRAM	MGIA	MRAM	MRAM	MGIA	MRAM
0	0600	0	0	0	0	0	0	0	0	0
3		0	3	3	3	6	6	6	6	6
6		0	*[4]	*	3	*	*	5	*	*
9	1500	0	0	0	2	6	6	2	6	6
15	0600	*	*	*	0	4	4	0	4	6
24		0	0	0	0	0	0	0	0	2

[1]n = 3.

[2]n = 6.

[3]Feeding of spiked milk replacer occurred following the collection of serum and urine samples.

[4]No samples taken.

cm square sections and placed on ice. Serum and tissue samples were stored at −75°C until assayed. Urine samples were collected from the bladder, stored on ice, and assayed with the MGIA and the MRAM within 6 h of slaughter.

RESULTS

Determination of penG and Amoxicillin in Serum and Urine by MRAM

Precision. For penG, the coefficients of variation were 5.2, 6.1, 7.5, 6.3, and 7.2% in serum and 4.6, 5.7, 8.6, 9.5, and 5.1% in urine for the 0, 0.006, 0.015, 0.03, and 0.06 μg of penG/ml standards, respectively. For amoxicillin, the coefficients of variation were 2.6, 5.2, 4.0, 5.7, and 9.7% in serum and 4.6, 7.1, 5.3, 7.8, and 6.7% in urine for 0, 0.01, 0.025, 0.05, and 0.10 μg/ml amoxicillin standards, respectively.

Accuracy. With the standard curve to determine measured penG concentrations, a significant ($P < 0.01$) correlation ($r = 0.98$) existed between known and measured penG concentrations. Regression analysis indicated that 97% of the variation between known and measured penG concentrations was explained by a linear relation. Predicted mean penG concentrations in serum of 0.001, 0.006, 0.017, and 0.033 μg/ml and in

Table 2. Detection of amoxicillin in serum and urine of calves after being fed milk replacer containing amoxicillin at 0.25, 1.0, and 2.00 μg/ml (ppm). Serum was assayed using a microbial receptor assay method (MRAM). The urine was assayed using the microbial growth inhibition assay (MGIA) and MRAM. The table indicates the number of individual calf serum or urine samples that had detectable levels of amoxicillin by MRAM and the number of individual calf urine samples that had positive MGIA results. A positive MGIA signifying a possible violative amoxicillin residue in the animal.

Sampling time after feeding (h)	Time of feeding spiked milk replacer[3]	0.25 μg/ml[1]			1.0 μg/ml[2]			2.0 μg/ml[2]		
		Serum	Urine		Serum	Urine		Serum	Urine	
		MRAM	MGIA	MRAM	MRAM	MGIA	MRAM	MRAM	MGIA	MRAM
0	0600	0	0	0	0	0	0	0	0	0
3		3	0	6	2	2	6	5[2]	5[2]	5[2]
6		1	*[4]	*	1	*	*	3	*	*
9	1500	0	0	6	0	2	6	3	*	*
15	0600	0	0	2	0	2	4	*	*	*
24		0	0	0	0	0	0	0	0	0

[1]n = 3.

[2]n = 6.

[3]Feeding of spiked milk replacer occurred following the collection of serum and urine samples.

[4]No samples taken.

Table 3. Dectection of penicillin G in serum and urine of calves after being fed milk replacer containing 11.7 μg/ml (ppm) of penicillin G (spiked with procaine penicillin G at 20.0 μg/ml). Serum was assayed by a microbial receptor assay method (MRAM). The urine was assayed by the microbial growth inhibition assay (MGIA) and MRAM. The table indicates the number of individual calf serum or urine samples that had detectable levels of penicillin G by MRAM and the number of individual calf urine samples that had positive MGIA results. A positive MGIA signifying a possible violative drug residue in the animal.

Sample time from the start of the trial (h)	Time of feeding the spiked milk replacer[1]	Sample time after a feeding (hours)	11.7 μg/ml		
			Serum[2]	Urine[2]	
			MRAM	MGIA	MRAM
0	0600	0	0	0	0
3		3	6	6	6
6		6	5	*[3]	*
9	1500	9	5	6	6
24	0600	15	0	5	6
27		3	5	5	6
30		6	3	*	*
33	1500	9	2	5	6
48	0600	15	0	5	5
51		3	6	6	6

[1]Feeding of spiked milk replacer occurred following the collection of serum and urine samples.
[2]n = 6.
[3]No samples taken.

urine of 0.001, 0.007, 0.014, and 0.027 μg/ml were measured for the 0, 0.006, 0.015, and 0.03 μg of penG/ml concentrations, respectively.

With the standard curve to determine measured amoxicillin concentrations, a significant ($P < 0.01$) correlation ($r = 0.98$) existed between known and measured amoxicillin concentrations. In serum and urine, regression analysis indicated that 97 and 95%, respectively, of the variation between known and measured amoxicillin concentrations was explained by a linear relation. Predicted mean amoxicillin concentrations in serum of 0.001, 0.011, 0.033, and 0.054 μg/ml and in urine of 0.002, 0.012, 0.025, and 0.048 μg/ml were measured for the 0, 0.01, 0.025, and 0.05 μg of amoxicillin/ml concentrations, respectively.

Between day precision. For both penG and amoxicillin, the day-to-day variability of the assay with either of the matrices was not significantly different.

Limit of detection. The B/B_0 values (mean ± SD) for serum and urine containing no penG were 0.94 ± 0.040 and 0.98 ± 0.045, respectively. A LOD of 0.005 μg/ml of penG was determined for the assay using either matrix. The B/B_0 value (mean ± SD) for serum and urine containing no amoxicillin was 0.97 ± 0.037 and 1.03 ± 0.061, respectively. A LOD of 0.006 and 0.013 μg of amoxicillin/ml for serum and urine, respectively, were determined for the assay.

Cross-reactivity. Serum samples with penG and cloxacillin differed significantly ($P < 0.01$, by use of least significant difference t test) from penG controls. There were no significant differences between the control penG samples (0.015 μg/ml) and the control penG samples containing sulfadimethoxine, oxytetracycline, or erythromycin.

Control amoxicillin samples (0.025 μg/ml) with the addition of cephapirin differed significantly ($P < 0.01$, by use of least significant difference t test) from amoxicillin controls. In the presence of the added cephapirin, the MRAM measured a concentration significantly greater than 0.025 μg of amoxicillin/ml. There were no significant differences between the amoxicillin controls and amoxicillin controls with the addition of sulfadimethoxine, oxytetracycline, or erythromycin.

There was a significant ($P < 0.01$) difference between amoxicillin controls (0.01 μg/ml) and samples with a combination of amoxicillin and penicillin at 0.01 and 0.006 μg/ml, respectively. There was a significant ($P < 0.01$) difference between penicillin controls (0.006 μg/ml) and samples with a combination of amoxicillin and penicillin at 0.01 and 0.006 μg/ml, respectively. The samples containing both β-lactams resulted in measurements of concentrations significantly greater than the 0.006 or 0.01 μg/ml of penicillin or amoxicillin, respectively.

Experiment 1: Antibiotic Residues in Urine and Serum Determined by MRAM

No urine or serum sample collected at time 0 tested positive for antibiotics by either the MGIA or the MRAM.

Results for penG are presented in Table 1. Regardless of the concentration of penG in the milk replacer, penG was detected by the MRAM in all urine samples from

calves 3 h after consuming milk replacer; the MGIA detected possible violative drug residues in the urine of calves 3 h after consuming milk replacer. Three hours after drinking milk replacer spiked at 0.293, 2.93, and 5.85 μg/ml (ppm), penG was detected in zero of three, three of six, and six of six serum samples, respectively. Data from three of the calves fed milk replacer spiked with 0.293 μg of penG/ml was inadvertently lost. Except for two of six urine samples from calves consuming milk replacer with 5.85 μg of penG/ml, penG could not be detected in serum or urine by either assay 24 h after drinking spiked milk replacer; the two samples had very low levels of penG detected by the MRAM.

Results for amoxicillin are presented in Table 2. Amoxicillin was detected in some serum samples 3 h after milk replacer was drunk, regardless of the concentration of amoxicillin in the milk replacer. With the MRAM, amoxicillin was detected in the urine from all tested calves at the 3-h sampling for all concentrations tested; the MGIA detected possible violative drug residues in some calves drinking milk replacer with 1.0 and 2.0 μg of amoxicillin/ml. In both the serum and urine samples collected 24 h after feeding spiked milk replacer, amoxicillin was not detected by either assay.

Experiment 2: Confirmation of PPG Residues in Tissue by HPLC

Detection of penG in serum and urine of calves fed milk replacer spiked with 11.7 μg of penG/ml (PPG at 20.0 μg/ml) is given in Table 3. Three hours after the initial feeding of spiked milk replacer, penG was detected by both assays in the serum and urine samples of all six calves. Fifteen hours after the calves drank spiked milk replacer, MGIA results on urine were positive for penG residues in the majority (five of six) of the calves. At slaughter, which occurred 51 h after the start of the trial, all urine samples, collected 3 h after the final feeding of spiked milk replacer, were positive for the presence of antimicrobial residues by the MGIA, and penicillin was detected by the MRAM. All serum samples, collected 3 h after the final feeding, had detectable levels of penG. Individual values of penG concentrations in serum and tissue samples, as determined by the HPLC assay, were used to calculate the mean (± SD) concentrations for the various samples (Table 4). The mean (± SD) penG concentrations measured in liver, kidney, muscle, and serum were 0.409 (±0.167) μg/g, 0.031 (±0.012) μg/g, 0.008 (±0.002) μg/g, and 0.013 (±0.006) μg/ml, respectively.

Table 4. Penicillin G concentrations in tissue (μg/g) and serum (μg/ml) measured in calves slaughtered 3 h after drinking milk replacer containing 11.7 μg/ml of penicillin G (spiked with procaine penicillin G at 20.0 μg/ml). Concentrations were determined by HPLC.

	Liver	Kidney	Muscle	Serum
Calf1	0.315	0.020	0.007	0.017
Calf2	0.514	0.039	0.006	0.020
Calf3	0.503	0.013	0.007	0.014
Calf4	0.241	0.034	*[1]	0.006
Calf5	0.640	0.046	0.011	0.017
Calf6	0.239	0.031	*	0.006
Mean	0.409	0.031	0.008[1]	0.013
SD	0.167	0.012	0.002[2]	0.006

[1]Below the limit of detection (LOD) for the HPLC method. LOD was 0.005 μg/g.

[2]Mean and standard deviation determined excluding samples below the limit of detection.

DISCUSSION

Because of the prominence of procaine penG in the treatment of cattle (Sundlof et al., 1995) and as a detected residue in marketed meat (Gibbons et al., 1996, Paige and Pell, 1997), penG was investigated in this study. Penicillin is administered to lactating cattle by the intravenous, subcutaneous, intramuscular, and intramammary routes. This process results in milk not acceptable for human consumption, also known as "waste" milk, which may be fed to calves. In one survey, β-lactam antibiotic residues were found in 46% of samples of fluid milk fed to calves (Selim and Cullor, 1997). Because penG has limited oral availability (Nathwani and Wood, 1993, Knifton, 1982), the authors decided to also examine a β-lactam with better oral availability, amoxicillin. Amoxicillin, a semisynthetic aminopenicillin, has been formulated to provide enhanced oral absorption compared with penG. When administered orally with milk replacer, amoxicillin had a bioavailability of approximately 30% in calves (Ziv et al., 1977). Amoxicillin is used in the treatment of lactating cattle, albeit to a lesser extent than penG (Sundlof et al., 1995).

The MGIA was used in this study to determine the possibility of potential violative drug residues. The MGIA is used to detect antimicrobials in urine of cattle and is a commercial product available to both veterinarians and producers. Animals with urine samples that cause a zone of inhibition of the *Bacillus subtilis* culture are classified as potentially having violative antibiotic residues present in edible tissues (Performing the live animal swab test: a self-instructional guide. 9/89. Editek, Inc., Burlington, NC).

The MRAM detects β-lactam antibiotics in milk, serum, and tissue. The assay was able to accommodate the numerous samples tested in this study and to provide results in a relatively timely manner. The assay had adequate precision and accuracy within established limits (Shah et al., 1992). Non β-lactam antibiotics did not interfere with the assay's performance, confirming the reported specificity of the MRAM to β-lactam antibi-

otics (Boison et al., 1995; Charm II test for beta-lactams in serum and tissue using tablet reagents—operator's manual. Charm Sciences Inc). The group-specific receptor used in the MRAM is specific between antibiotic families, but not within families. Our finding of activity by cloxacillin in the β-lactam MRAM was different than another study (Boison et al., 1995). In that study, cloxacillin was tested at a concentration of 0.05 μg/ml. The group specific receptor of the β-lactam MRAM has a lower affinity for cloxacillin; the level of detection for cloxacillin in milk is 0.07 μg/ml compared with 0.0048 μg/ml for penG (Anonymous, 1995). The higher concentration used in our study overcame the low affinity causing cloxacillin to react with the assay. With the recognition of its limitations, the MRAM is a convenient tool with which to detect and monitor the depletion of penG in serum, a finding that is consistent with another published report (Boison et al., 1995).

At the concentrations studied, amoxicillin and penG had the potential to cause short-term drug residues. The detection of both β-lactams in serum and urine, even at the lower doses, suggested the potential exists for violative drug residues. The lower doses examined were to approximately simulate the low range of concentrations that could occur following treatment with one intramammary infusion syringe (labeled dose). These findings are similar to a previous study that fed milk replacer with 6.6 and 13.2 μg/ml of penG (Prange et al., 1984). Because calves marketed through livestock auctions are often processed within 12 h (Wiggers and Wilson, 1996), the positive MGIA results seen in calves 15 h after drinking milk replacer with either amoxicillin or penicillin suggest that violative drug residues could occur in marketed calves.

Because the MRAM and MGIA results detected antibiotic residues in calves and suggested possible violative drug residues in tissues, penG concentrations were measured in the tissues of calves 3 h following the consumption of milk replacer containing PPG at 20.0 μg/ml (11.7 μg of penG/ml). A 3-h slaughter time was chosen to test tissues close to maximum concentration. Peak plasma concentrations for similar β-lactam antibiotics occurred at approximately 1.5 to 3 h (Palmer et al., 1983; Soback et al., 1987; Ziv et al., 1977).

Penicillin G concentrations in the liver were 5- to 12-fold greater than the established tolerance level in edible tissue, 0.050 μg/g (Table 4). This demonstrates that violative drug residues may occur following the consumption of milk contaminated with penG. In previous studies with microbial growth inhibition tests, drug residues were not detected (Duby et al.; 1984, Prange et al., 1984). However, kidney and muscle were the only tissues examined. Slaughter times were 14 to 18 h (Duby et al., 1984) and 16 h after feeding diluted milk with penG (Prange et al., 1984). The greater time period between drinking spiked milk replacer to slaughter and the fact liver was not tested for violative drug residues may have contributed to residues not being detected in the other studies (Duby et al., 1984; Prange et al., 1984). In a study on the depletion of penG residues in yearling steers after parenteral administration, the liver had greater concentrations of penG than did the kidney (Korsrud et al., 1993); this finding was also reflected in our study. Neither kidney nor muscle had penG concentrations above the established tolerance level, although kidney concentrations were very close to the tolerance level. Our results, indicating the highest concentrations of penG were in liver with muscle concentrations approximately 1/4 to 1/3 of serum concentrations, were similar to previous studies (Korsrud et al., 1993).

CONCLUSION

We conclude that milk replacer with penG or amoxicillin caused detectable levels in the urine, and the urine tested positive for possible violative residues using the MGIA. Violative drug residues in liver were found 3 h after calves consumed milk replacer with 11.7 μg/ml of penG; however, drug residues in muscle at that time were at least 5-fold or more below the tolerance level for penG. Twenty-four hours after the feeding of milk replacer with penG or amoxicillin was discontinued, all urine samples tested negative by the MGIA. These results indicated that milk replacer or milk containing penG or amoxicillin, at the levels tested, has the potential to produce short-term residues when fed to calves.

Further research examining drug residues in different tissues over an extended period needs to be performed. Until more definitive studies are completed, this study suggests it would be prudent to refrain from marketing calves recently fed milk from cows treated with β-lactam antibiotics for at least 24 h. In a related situation, the Food Animal Residue Avoidance Databank has recommended a withdrawal time of 4 d when calves are fed colostrum from cows having been treated during the dry period, 26 d prior, with an intramammary infusion of 1 million units of PPG per quarter (Rangel-Lugo M et al., 1998). The results of this investigation, along with Food Animal Residue Avoidance Databank recommendations, may be useful in determining appropriate slaughter withholding periods necessary to prevent residues in calves feed milk containing penicillin or amoxicillin. Feeding nonmedicated milk replacer or milk from untreated cows before marketing calves will reduce the possible occurrence of residues due to feeding waste milk.

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